Brief Report

A Functional Polymorphism in the COMT Gene and Performance on a Test of Prefrontal Cognition

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Objective: In the prefrontal cortex, the enzyme catechol *O*-methyltransferase (COMT) is critical in the metabolic degradation of dopamine, a neurotransmitter hypothesized to influence human cognitive function. The COMT gene contains a functional polymorphism, *Val158Met,* that exerts a fourfold effect on enzyme activity. The current study investigated whether prefrontal cognition varies with COMT genotype.

Method: *Val158Met* was genotyped in 73 healthy volunteers. A task of prefrontal cognition, the Wisconsin Card Sorting Test, was also administered.

Results: Subjects with only the low-activity *met* allele made significantly fewer perseverative errors on the Wisconsin Card Sorting Test than did subjects with the *val* allele.

Conclusions: These data are consistent with those of previous studies, suggesting that a functional genetic polymorphism may influence prefrontal cognition.

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Several lines of evidence suggest that the neurotransmitter dopamine plays an important role in human cognition. Computational modeling studies indicate that dysfunction in dopamine systems accounts for abnormal cognitive control in the prefrontal cortex (1). In laboratory animals, low transmission of dopamine in the prefrontal cortex is associated with impairments in cognitive performance (2), and in humans, pharmacological enhancement of dopaminergic activity can produce improvements in specific cognitive domains dependent on the integrity of the prefrontal cortex (3).

The major mechanism by which the synaptic activity of dopamine is terminated is reuptake, followed by metabolic degradation. Catechol *O*-methyltransferase (COMT) is the major mammalian enzyme involved in the metabolic degradation of released dopamine and accounts for more than 60% of the metabolic degradation of dopamine in the frontal cortex (4). It is therefore plausible that genetic factors that affect COMT function may significantly influence cognition through effects on dopaminergic function.

The COMT gene contains a functional polymorphism that codes for a substitution of methionine (*met*) for valine (*val*) at codon 158 (5). The *met* allele is thermolabile and has one-fourth the enzymatic activity of the *val* allele (5). Recently, Egan and colleagues (6) reported that subjects with the low-activity *met* allele performed better (i.e., had fewer perseverative errors) on a neurocognitive test, the Wisconsin Card Sorting Test (7), than subjects with the *val* allele. This relationship of genotype to cognitive performance was observed in three groups of subjects of European origin: healthy volunteers (N=55), schizophrenia patients (N=175), and the siblings of schizophrenia patients (N=219). The finding of a significant relationship between COMT genotype and cognitive function in healthy subjects requires replication. Therefore, in the present study we examined a cohort of healthy volunteers who took the Wisconsin Card Sorting Test and were genotyped at the COMT *Val158Met* locus to test the hypothesis that subjects with only the COMT *met* allele would perform better on the Wisconsin Card Sorting Test than subjects with the COMT *val* allele.

Method

The participants were 73 healthy volunteers, 42 men and 31 women, with a mean age of 31.3 years (SD=10.2). There were 49 Caucasians, 14 blacks, five Hispanics, three Asians, and two with mixed ethnicity. All provided written informed consent. All subjects were free of psychiatric disorders, as determined by a structured diagnostic interview, and in good physical health, as determined by physical examination, electrocardiogram, and laboratory tests including liver and thyroid function tests and urinalysis. All had been free of drug and alcohol abuse for at least 6 months.

The subjects were each assessed with the Wisconsin Card Sorting Test, a widely used measure of prefrontal cognitive function that is sensitive to a subject's ability to generate hypotheses, establish response sets, and fluently shift sets. Subjects are required to sort stimulus cards on the basis of perceptual attributes (color, form, number). The only feedback provided by the administrator is whether each response is correct or incorrect. The sorting rule is changed after 10 consecutive correct responses. Testing is discontinued when the subject has learned two iterations of the three sorting rules or has reached 128 trials (7). The primary outcome measure used for this study was the number of perseverative errors, a measure sensitive to an individual's ability to fluently shift cognitive sets and the measure used by Egan and colleagues (6).

COMT *Val158Met* genotypes were determined by restriction fragment length polymorphism. A 109-base-pair polymerase

chain reaction (PCR) product was generated in 30 cycles with an annealing temperature of 54°C by using the primers Comt1 nt 1881 5′ CTCATCACCATCGAGATCAA and Comt2 nt 1989 5′ CCAGGTCTGACAACGGGTCA.

The *val* and *met* alleles were discriminated by digesting the PCR product with N1aIII at 37°C for 4 hours, followed by 4.5% agarose gel electrophoresis. The *val/val* homozygotes (86 and 23 base pairs), *met/met* homozygotes (68 and 18 base pairs), and *val/met* heterozygotes (86, 68, 23, and 18 base pairs) were visualized by ethidium bromide staining.

Welch's analysis of variance (ANOVA) was carried out with COMT genotype as the independent factor and number of perseverative errors as the dependent measure (8). This procedure was used because a test of the assumption of homogeneity of variance indicated that the variances within the groups were dissimilar ($F=3.42$, df=2, 70, $p=0.04$), and Welch's ANOVA is robust to such violations. A mixed-model analysis that fit separate variances for each group was then used to conduct paired comparisons.

Results

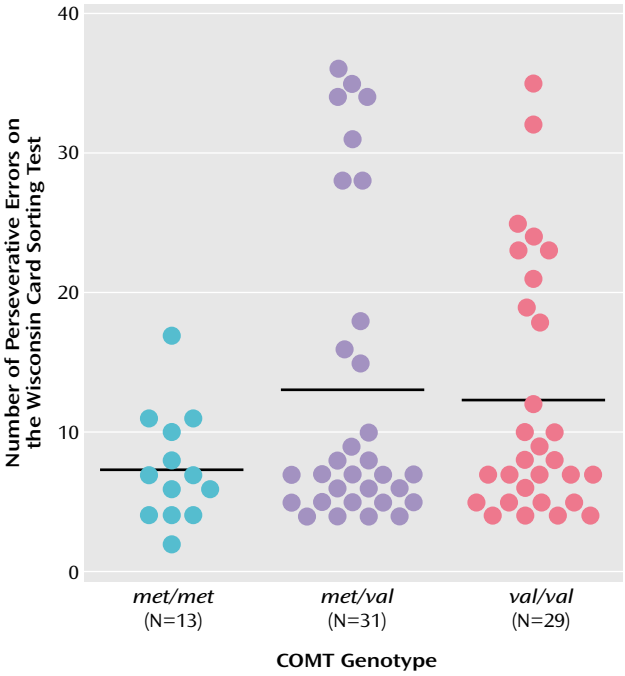
The results are displayed in Figure 1. There were 13 subjects with the met/met COMT genotype, 31 with the met/val genotype, and 29 with the val/val genotype, a distribution consistent with Hardy-Weinberg expectations ($\chi^2=0.84$, df=2, $p=0.67$). The mean number of categories completed by the 73 subjects was 5.48 (SD=1.28), and there was no significant difference ($F=1.44$, df=2, 70, $p=0.25$) between the three genotypic groups. The mean number of perseverative errors for the subjects with the met/met genotype was 7.46 (SD=4.01), for met/val it was 13.03 (SD=11.18), and for val/val it was 12.21 (SD=9.08). ANOVA revealed a significant relationship between COMT Val158Met genotype and the number of perseverative errors ($F=4.43$, df=2, 70, $p=0.02$). The met/met group committed significantly fewer perseverative errors than either the met/val group ($t=2.43$, df=70, $p=0.02$) or the val/val group ($t=2.35$, df=70, $p=0.02$). There was no significant difference between the performances of the met/val and val/val groups ($t=0.31$, df=70, $p=0.75$). No significant differences were observed between the three genotypic groups in age ($F=0.03$, df=2, 70, $p=0.97$), sex ($\chi^2=4.67$, df=2, $p=0.10$), or ethnicity (Fisher's exact test, $p=0.18$).

Discussion

The present study tested the relationship between COMT *Val158Met* genotype and performance on the Wisconsin Card Sorting Test in healthy subjects. Subjects with the *met/met* genotype performed significantly better than *met/val* and *val/val* subjects on the outcome variable, perseverative errors.

These data are consistent with the results of other studies examining the role of COMT in cognitive function. First, Egan and colleagues (6) recently reported that healthy subjects with the *met* allele produced fewer perseverative errors on the Wisconsin Card Sorting Test than subjects with the *val* allele. Moreover, COMT gene knockout mice display better memory than wild-type mice under conditions of environmental stress (9). Finally, clinical trials data (3) indicate that pharmacological inhibition of COMT function with the antiparkinsonian agent tolcapone improves cognitive performance. Taken together, these data provide evidence that reductions in COMT function, whether induced by a pharmacological agent, gene knockout, or the presence of a low-function allele, are associated with improved cognitive performance.

FIGURE 1. Perseverative Errors on the Wisconsin Card Sorting Test of Healthy Volunteers Categorized by Genotype at the *Val158Met* Locus of the Gene for Catechol *O*-Methyltransferase (COMT)[a]



[a] The horizontal lines represent mean values.

Several other factors should be considered in the interpretation of these data. Performance on the Wisconsin Card Sorting Test may vary among ethnic groups, and this result could reflect undetected ethnic differences between phenotypic groups. However, analysis of only the Caucasian subjects within the present data set revealed the same pattern of results seen in the larger group. Among the Caucasians, the *met/met* subjects produced 23% fewer perseverative errors (mean=7.58, SD=4.17) than the *met/val* subjects (mean=9.87, SD=9.43), and 17% fewer perseverative errors than the *val/val* subjects (mean=9.08, SD=6.47). These differences are smaller than the differences observed in the study group as a whole, which suggests that the effect of the COMT *Val158Met* polymorphism is stronger in some ethnic groups than in others. In addition, it should be noted that the study by Egan and colleagues (6) used Caucasian subjects of European origin, whereas the Caucasian subjects in our study were not classified by ancestral origin, so these data are not precisely comparable. Quantitative family-based association studies (10) or assessment of potential stratification with genomic control techniques (11) (when

available for quantitative traits) will be helpful in resolving this issue in future studies. The COMT *Val158Met* polymorphism could be in linkage disequilibrium with another variant, and the associations between *Val158Met* and performance on the Wisconsin Card Sorting Test may reflect the influence of these other variants. However, the COMT gene has been extensively studied, and to our knowledge, no other functional variant in linkage disequilibrium with *Val158Met* has yet been identified (12). Indeed, we elected to test the only known functional polymorphism within the COMT gene, and therefore the polymorphism with the highest a priori probability of influencing a cognitive phenotype, rather than genotyping additional markers and diffusing the power of this study to detect a functional signal. If larger data sets become available, further study of this gene and performance on the Wisconsin Card Sorting Test may be warranted.

This association between the COMT *Val158Met* polymorphism and performance on a test of executive cognitive function is consistent with the findings in the study of this polymorphism by Egan et al. (6) and provide what we believe to be the first independent support for a role of COMT genotype on one aspect of prefrontal cognition.

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